European Journal of Endocrinology (2002) **146** 75–79 ISSN 0804-4643

CASE REPORT

Early postnatal treatment of hypogonadotropic hypogonadism with recombinant human FSH and LH

Katharina M Main, Ida M Schmidt, Jorma Toppari[1] and Niels E Skakkebæk

University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark and [1]Departments of Pediatrics and Physiology, University of Turku, Turku, Finland

(Correspondence should be addressed to K M Main, Department of Growth and Reproduction, Section 5064, The National University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark; Email: RH04639@RH.DK)

Abstract

Background: Patients with hypogonadotropic hypogonadism may be diagnosed shortly after birth because of micropenis and cryptorchidism, combined with subnormal LH and FSH concentrations during the postnatal period.
Objective: To investigate whether treating these patients with gonadotropins postnatally, to mimic the physiological development, would improve testicular growth and fertility potential later in life.
Design: Our patient presented with micropenis. Serum hormone concentrations were measured monthly after delivery: LH and testosterone were undetectable, and FSH and inhibin B were below the normal range (0.05–0.17 IU/l and 79–112 pg/ml respectively).
Methods: From 7.9 to 13.7 months of age, the patient was treated with recombinant human LH and FSH in doses of 20 and 21.3 IU s.c. twice weekly respectively.
Results: During treatment concentrations of LH, FSH, inhibin B and estradiol increased to values within normal limits (0.7–1.88 IU/l, 0.17–3.24 IU/l, 121–268 pg/ml and 40–55 pmol/l respectively), whereas serum testosterone remained undetectable. Penile length increased from 1.6 to 2.4 cm and testicular volume, assessed by ultrasound, increased by 170%. No significant adverse events were observed.
Conclusions: Gonadotropin treatment in an infant with hypogonadotropic hypogonadism succeeded in inducing an increase in inhibin B and testicular growth.

European Journal of Endocrinology **146** 75–79

Introduction

Patients with hypogonadotropic hypogonadism may present at birth with symptoms of micropenis or maldescended testes, or both. These boys lack the early postnatal surge in reproductive hormones that is normally seen in healthy boys – a feature that can be used for early establishment of the diagnosis (1, 2).

In addition to androgen replacement therapy during puberty and adulthood, fertility is becoming an increasingly important aspect of patient care, as possibilities of treating male infertility improve. To date, mainly adolescents and adult patients have been treated with monotherapy or combinations of human chorionic gonadotrophin (hCG), human MG and follicle-stimulating hormone (FSH) (3–7). Results depend on pre-treatment testicular size and whether or not there has been cryptorchidism. Prepubertal treatment with recombinant FSH stimulates inhibin B production and testicular growth (8).

Gonadotropins and androgens have a role in Leydig cell proliferation and germ cell differentiation early postnatally (9). Thus treatment of hypogonadotropic hypogonadism with gonadotropins during the postnatal period, when spontaneous production of reproductive hormones is high in healthy boys, may induce testicular growth and thereby improve future fertility potential. We present the first results of recombinant gonadotropin treatment in a boy with hypogonadotropic hypogonadism.

Case report

Patient

This was the third child of consanguinous Turkish parents. There was a family history of pes equinovarus and congenital heart disease, but no reproductive disorders. The patient was referred at the age of 2 weeks because of micropenis (1.6 cm stretched length). Both testes were fully descended and the scrotum well developed. The karyotype was 46,XY. Monthly measurements of spontaneous serum concentrations of reproductive hormones over 6 months revealed a total

lack of luteinizing hormone (LH) and testosterone. FSH and inhibin B were persistently below the normal range for his age (10) (Table 1). hCG stimulation with 100 IU/kg (single dose) increased serum testosterone to 5.7 nmol/l. All other pituitary axes were found to be normal. Thus the diagnosis of hypogonadotropic hypogonadism was established. Psychomotor development during follow-up until 1.5 years has been normal.

Treatment

At the age of 7.9 months, the child began treatment with recombinant human LH and FSH (treatment schedule outlined in the Table 1) after an initial kinetic study with s.c. injection of 20 IU LH and 2.5 IU/kg FSH (total dose: 21.3 IU). The dose of LH was doubled at 9.6 months. At the age of 12.2 months, testosterone suppositories were administered to improve penile growth further, and LH treatment was stopped. FSH injections were suspended at 13.7 months. The injections were given by a pediatric nurse as separate injections for LH and FSH, alternating between right and left thigh and gluteal region. The treatment was generally well tolerated and compliance excellent. No serious adverse events were encountered. There was recurrent otitis media throughout the treatment period, sleep disturbances between weeks 2 and 5 of treatment, intermittent nausea, and a local rash once at the injection site, which resolved spontaneously. Body hair and pigmentation increased slightly in amount during treatment. Control blood samples (hematology, liver and kidney function, coagulation status, thyroid hormones, insulin-like growth factor (IGF)-I and IGF-I binding protein-3) remained normal.

Drugs

Recombinant human LH and FSH (Luveris 37.5 IU and Gonal-F 37.5 IU) were obtained from Ares Serono, Geneva, Switzerland. Before injection, LH was dissolved in 0.5 ml solvent, and FSH in 1 ml solvent. Testosterone suppositories (1 mg) were manufactured by the hospital's pharmacy, using a modification of the method of Hamburger & Pedersen-Bjergaard (11). This treatment is an effective alternative to peroral and injection therapy for androgen replacement (12).

Assays

Serum LH, FSH and sex hormone binding globulin (SHBG) were measured by time-resolved immunofluorimetric assays (Delfia, Wallac Inc., Turku, Finland). Detection limits were 0.06 IU/l, 0.05 IU/l and 0.23 nmol/l respectively. Intra- and interassay coefficients of variation were less than 8% for LH and FSH, and 5% for SHBG. Serum testosterone was measured by RIA (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA, USA) with a detection limit of 0.23 nmol/l and intra- and interassay coefficients of variation less than 10%. Serum inhibin B was measured by specific ELISA (13) with a detection limit of 20 pg/ml, the intra- and interassay coefficients of variation were 15% and 18% respectively. Serum estradiol was determined by RIA (Pantex, Santa Monica, CA, USA) with a detection limit of 18 pmol/l and intra- and interassay coefficients of variation less than 8% and 13% respectively.

Ultrasound

Testis volume was determined with ultrasound (Aloka Micrus, Tokyo, Japan, linear transducer 7.5 MHz), and calculated as the mean volume of the left and right sides (14).

Normal values

Measurements were compared with age-specific longitudinal values for healthy boys (10), normal 95% ranges at 3 months being: testosterone 0.43–7.71 nmol/l, inhibin B 193–563 pg/ml, FSH 0.86–2.52 IU/l, LH 0.65–2.69 IU/l, estradiol 18.0– 44.4 pmol/l.

Table 1 Treatment schedule and serum concentrations of reproductive hormones (minimum and maximum values) during treatment of an infant with hypogonadotropic hypogonadism with recombinant human LH and FSH and testosterone suppositories. In treatment period 3, only one sample was available. Blood samples were taken before, and 4 and 6 h after injection.

	Pretreatment	Period 1	Period 2	Period 3
Age (months)	0–7.9	7.9–9.6	9.6–11.3	12.2–13.7
Total LH dose (IU × 2/week)		20	40	
Total FSH dose (IU × 2/week)		21.3	21.3	21.3
Testosterone suppositories (mg/day)				1
Testosterone (nmol/l)	n.d.	n.d.	n.d.	3.3
LH (IU/l)	n.d.	0.7–0.89	1.3–1.88	n.d.
FSH (IU/l)	0.05–0.17	0.17–2.12	1.26–3.24	0.88
SHGB (nmol/l)	123–169	150–176	125–150	153
Estradiol (pmol/l)	25–27	40	55	26

n.d. = not detectable.

Ethics

The treatment was commenced after written consent from both parents, consent from the local Ethics Committee (07-00-013/00) and permission from the Danish Ministry of Health (2512-8928, 2512-10089).

Results

A single injection of 20 IU LH and 21,3 IU FSH s.c. increased serum LH and FSH to within normal limits (Fig. 1).

Results of serum measurements of reproductive hormones (minimum and maximum values) before and during the different treatment periods are shown in Table 1. LH and FSH increased to values within the low- and high-normal ranges respectively. Testosterone remained unmeasurable throughout the period, until testosterone suppositories were added to the treatment. Estradiol values increased to the high-normal range. After doubling of the dose of LH, an increased value was measured once. No gynecomastia developed. SHBG measurements remained unaltered. Serum inhibin B concentrations increased to approximately double the basal value during treatment with FSH and LH (Fig. 2). Addition of testosterone in period 3 caused a further increase.

The increase in serum inhibin B was parallel to the increase in testicular volume, as determined by ultrasound (Fig. 2). Testicular volume decreased from 59 mm^3 at birth to 31 mm^3 at 7 months of age. After 5 months of LH and FSH treatment, testicular volume had increased to 84 mm^3 (+170%). During LH and FSH treatment and before the start of testosterone treatment, stretched penile length increased from 1.6 cm to 2.4 cm. After supplementary treatment with testosterone, penile length was 2.7 cm.

During LH and FSH treatment, the standard deviation score for body height remained constant at −1.0, and bone age was equivalent to chronological age at 14 months. The weight-for-height percentile remained constant, at 60–65.

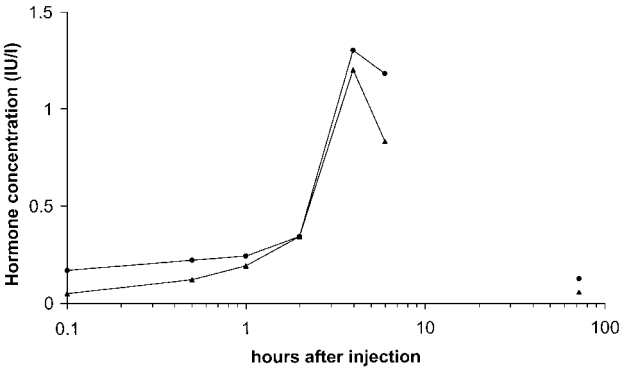

Figure 1 Serum LH (▲) and FSH (●) after a single s.c. injection of recombinant human LH (20 IU) and FSH (21.3 IU) in a 7-month-old infant with hypogonadotropic hypogonadism.

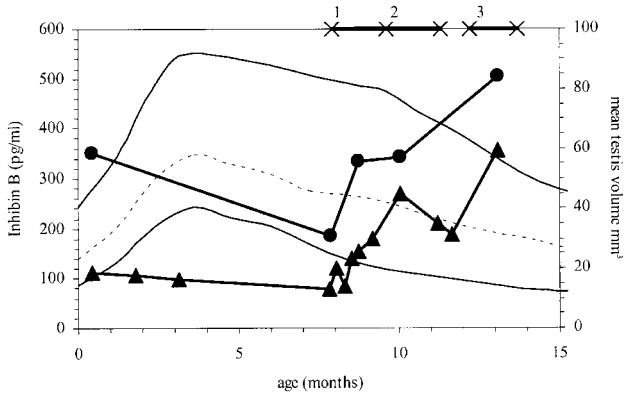

Figure 2 Serum inhibin B values (▲) and testicular volume (●) before/during treatment of an infant with hypogonadotropic hypogonadism with recombinant human FSH and LH and testosterone suppositories. 1, 2, 3, treatment periods 1, 2 and 3. Normal values (median and 95% confidence limits) are shown as the dotted and thin solid lines respectively.

Discussion

This is, to our knowledge, the first report of a patient with hypogonadotropic hypogonadism treated with recombinant human LH and FSH during the first year of life. The treatment was successful in inducing testicular growth and, to some extent, in improving penile length. However, testosterone was also needed to achieve sufficient phallic growth.

Healthy boys have a significant increase in spontaneous production of reproductive hormones in the early period after birth (15–17). Animal studies and human observations suggest that this postnatal surge is important for genital growth and, potentially, also for functional development of the gonads (9, 18–20). There is evidence for a role of gonadotropins and androgens, not only during puberty but also postnatally, for Leydig cell proliferation and germ cell differentiation (9–21). Sertoli cell numbers increase in the first 3 months of life (22) and again during puberty, and the total number achieved is predictive of future spermatogenic potential. In prepuberty, the testis consists mainly of seminiferous tubules, the length of which correlates with Sertoli cell numbers (23). Leydig cell volume increases around 3 months of age, probably under the influence of the LH surge (24). The increase in testosterone is probably initiated by LH. However, in culture, FSH also can induce testosterone response, potentially through paracrine mechanisms mediated by the Sertoli cells (24). Thus it seems rational to mimic the physiological development by replacing gonadotropins early in life. Such treatment has two goals: to treat the clinical symptoms of the patient during infancy and to improve the potential for achieving fertility.

The treatment doses applied here were derived empirically. The initial pharmacokinetic study in this patient indicated that the dosage was sufficient to augment LH and FSH to the normal range for 3-month-old boys. The results were comparable to those from studies in adult volunteers, showing a significant increase in serum concentrations of FSH and LH within 12 h and a more rapid decline of LH than of FSH (25).

Control blood samples showed persistently low LH and unmeasurable testosterone values throughout treatment. This may indicate that the half-life of LH was too short to maintain measurable values, or that the dose of LH was too low. In addition, the assay for testosterone does not have sufficient sensitivity to permit detection of very low serum concentrations. There were, however, clinical observations suggesting that there may have been a significant increase in intratesticular testosterone: penile length increased and body hair became more pronounced – both phenomena are androgen-dependent. Estradiol concentrations increased slightly, especially after doubling of the LH dose, which could indirectly reflect increased testosterone concentrations. However, the accuracy of the estradiol assay for very low concentrations is not good enough to exclude random fluctuation. Testicular growth was also observed, and may have been caused by direct FSH stimulation of Sertoli cell proliferation or intratesticular testosterone stimulation of the seminiferous epithelium, or both.

The fact that hCG injection in our patient lead to an increase in serum testosterone, whereas injection of LH did not, may indicate that the underlying genetic mechanism is an LH receptor mutation (26). However, low LH concentrations are not in agreement with LH resistance. We based the diagnosis in this patient exclusively on measurement of hormonal values (1, 2). Another genetic mechanism may be a mutation in the gonadotropin-releasing hormone receptor (27). However, these mutations are usually found only in cases of familial hypogonadotropic hypogonadism.

The dose of FSH was effective in increasing both FSH and inhibin B concentrations to within normal limits, and the addition of testosterone further increased inhibin B values. This may simply be due to a time-lag between start of treatment, when the testicles had not been stimulated for 8 months, and the achievement of a maximum response. It may, however, also indicate a synergistic effect of testosterone and FSH in stimulation of inhibin B production and secretion. In adults, inhibin B concentrations in serum are negatively correlated with serum FSH concentrations, but this feedback mechanism cannot be found in the first 2 years of life (10). Sertoli cells may be capable of an autonomous production of inhibin B, once they have been stimulated.

In conclusion, treatment of hypogonadotropic hypogonadism in one infant with recombinant human FSH and LH induced testicular and penile growth and augmented serum concentrations of inhibin B, thus succeeding in mimicking the physiological development that occurs early after birth. It remains to be seen whether early replacement with gonadotropins will be beneficial for the reduced fertility potential often seen in adult patients with hypogonadotropic hypogonadism despite hormonal treatment.

Acknowledgements

Recombinant human LH (Luveris) and FSH (Gonal-F) were kindly donated by Ares Serono Inc. S.A., Geneva, Switzerland (LSF-4138/IMP-21707). We appreciate the skilful assistance of pediatric nurse, Lilian Jakobsen.

References

1 Main KM, Schmidt IM & Skakkebæk NE. A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4905–4907.

2 Evain-Brion D, Gendrel D, Boxxola M, Chaussain JL & Job JC. Diagnosis of Kallmann's syndrome in early infancy. *Acta Paediatrica Scandinavica* 1982 **71** 937–940.

3 Barrio R, de Luis D, Alonso M, Lamas A & Moreno JC. Induction of puberty with human chorionic gonadotropin and follicle-stimulating hormone in adolescent male with hypogonadotropic hypogonadism. *Fertility and Sterility* 1999 **71** 244–248.

4 European Metrodin HP Study Group. Efficacy and safety of highly purified urinary follicle-stimulating hormone with human chorionic gonadotropin for treating men with isolated hypogonadotropic hypogonadism. *Fertility and Sterility* 1998 **70** 256–262.

5 Fuse H, Akashi T, Kazama T & Katayama T. Gonadotropin therapy in males with hypogonadotropic hypogonadism: factors affecting induction of spermatogenesis after gonadotropin replacement. *International Journal of Urology and Nephrology* 1996 **28** 367–374.

6 Burgues S & Calderon M. Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotropic hypogonadism. Spanish Collaborative Group on Male Hypogonadotropic Hypogonadism. *Human Reproduction* 1997 **12** 980–986.

7 Bouvattier C, Tauber M, Jouret B, Chaussain J-L & Rochiccioli P. Gonadotropin treatment of hypogonadotropic hypogonadal adolescents. *Journal of Pediatric Endocrinology and Metabolism* 1999 **12** 339–344.

8 Raivio T, Toppari J, Perheentupa A, McNeilly AS & Dunkel L. Treatment of prepubertal gonadotrophin-deficient boys with recombinant human follicle-stimulating hormone. *Lancet* 1997 **350** 263–264.

9 Mann DR & Fraser HM. The neonatal period: a critical interval in male primate development. *Journal of Endocrinology* 1996 **149** 191–197.

10 Andersson A-M, Toppari J, Haavisto A-M, Petersen JH, Simell T, Simell O & Skakkebaek NE. Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 675–681.

11 Hamburger C & Pedersen-Bjergaard K. Om testosterone-suppositorier med særligt henblik på suppoitorie-masse. *Archives of Pharmacological Chemistry* 1961 **68** 146–151.

12 Aakvaag A & Vogt JH. Plasma testosterone values in different forms of testosterone treatment. *Acta Endocrinologica* 1969 **60** 537–542.

13 Groome NP, Illingworth PJ, O'Brien M, Cooke I, Ganesan TS, Baird DT & McNeilly AS. Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. *Clinical Endocrinology* 1994 **40** 717–723.

14 Lenz S, Gowercman A, Elsborg A, Cohr K-H & Jelnes JE. Ultrasonic testicular texture and size in 444 men from the general population: correlation to semen quality. *European Journal of Urology* 1993 **24** 231–238.
15 Winter JSD, Faiman C, Hobson WC, Prasad AV & Reyes FI. Pituitary–gonadal relations in infancy. 1. Patterns of serum gonadotropin concentration from birth to four years of age in man and chimpanzee. *Journal of Clinical Endocrinology and Metabolism* 1975 **40** 545–551.
16 Winter JSD, Hughes IA, Reyes FI & Faiman C. Pituitary–gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *Journal of Clinical Endocrinology and Metabolism* 1976 **42** 679–686.
17 Forest MG. Pituitary gonadotrophin and sex steroid secretion during the first two years of life. In *Control of the Onset of Puberty*, pp 451–477. Eds M-M Grumbach, P-C Sizonenko & M-L Aubert. Baltimore: Williams & Wilkins, 1990.
18 Sharpe R, Walker M & Millar MR. Effect of neonatal gonadotropin-releasing hormone antagonist administration on Sertoli cell number and testicular development in the marmoset: comparison with the rat. *Journal of Endocrinology* 2000 **149** 191–197.
19 Mann DR, Akinbami MA, Gould KG, Tanner JM & Wallen K. Neonatal treatment of male monkeys with a gonadotropin-releasing hormone agonist alters differentiation of central nervous system centers that regulate sexual and skeletal development. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 1319–1324.
20 Wallen K, Maestripieri D & Mann DR. Effects of neonatal testicular suppression with a GnRH antagonist on social behavior in group-living juvenile rhesus monkeys. *Hormones and Behaviour* 1995 **29** 322–337.
21 Nachtigall LB, Boepple PA, Seminara SB, Khoury RH, Sluss PM, Lecain AE & Crowley WF Jr. Inhibin B secretion in males with gonadotropin releasing hormone (GnRH) deficiency before and during long-term GnRH replacement: relationship to spontaneous puberty, testicular volume, and prior treatment – a clinical research center study. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3520–3525.
22 Cortes D, Müller J & Skakkebæk NE. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *International Journal of Andrology* 1987 **10** 589–596.
23 Rey RA, Campo SM, Bedecarrás P, Nagle CA & Chemes HE. Is infancy a quiescent period of testicular development? Histological, morphometric, and functional study of the seminiferous tubules of the Cebus monkey from birth to the end of puberty. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 1325–1331.
24 Codesal J, Regadera J, Nistal M, Regadera-Sejas J & Paniagua R. Involution of human fetal Leydig cells. An immunohistochemical, ultrastructural and quantitative study. *Journal of Anatomy* 1990 **172** 103–114.
25 Le Cotonnec JY, Loumaye E, Porchet HC, Beltrami V & Munafo A. Pharmacokinetics and pharmacodynamic interactions between recombinant human luteinizing hormone and recombinant human follicle-stimulating hormone. *Fertility and Sterility* 1998 **69** 201–209.
26 Gromoll J, Eiholzer U, Nieschlag E & Somino M. Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2281–2286.
27 Beranova M, Oliveira MB, Bédécarrats GY, Schipani E, Vallejo M, Ammini AC *et al.* Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutation in idiopathic hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1580–1588.

Received 11 May 2001
Accepted 19 September 2001